INTRODUCTION

Cells, the basic component of all living organisms, are sensitive to radiation. This sensitivity is recorder to differ between individuals. At a molecular level, damages in irradiated cells are immediate. They affect the cell’s genetic material. At a cellular level, effects are delayed and include death of cells, genomic instability in the progeny of irradiated cells, and other non-targeted effects. At the organism’s level, exposure to radiation induce development of tumors. This multistage process is long and complex, so cancers appear up to twenty years after irradiation.

X-rays, neutrons, alpha- and beta-particles come from natural environment or are produced by human activities. Apart from nuclear facilities (including fission and fusion reactors, radioactive waste repositories), industry and medicine (radiotherapy) also include utilization of radioactive products or radiations. Exposure to high level of ionizing radiation clearly leads to development of cancers. But effects on human health slow level of exposure to radiations can not be reliably assessed by epidemiological methods, nor is thoroughly understood by scientists.

Radiation – the word immediately evokes the dreadful fallout of nuclear bombs or the aftermath of the Chernobyl disaster. Yet radiation is also useful weapon in the therapeutic arsenal, for medical imaging as well as combating cancer. More subtly, it is also present in our everyday environment that is home to an increasing variety of ionizing radiation of natural or technological origin – but which is not considered a danger as it is far below the acceptable levels. The real long-term effects of this background radiation are nevertheless a mystery, which is why they are currently being investigated by the scientific projects.
RISC-RAD (Radiosensitivity of Individuals and Susceptibility to Cancer induced by Ionizing Radiations) is a fundamental research project in the field of radiobiology. This four-year project funded by the European Commission (EC) started on the 1st January 2004 and addresses the challenging issue of cancer risk assessment at low doses of ionizing radiation (RISC-RAD, 2007). In its 2005 brochure, Euratom Research Projects and Training Activities, the European Commission reports: “Radiation protection has always been a significant part of nuclear research and the understanding derived from these studies underlies the health and safety standards and exposure limits established today. However, understanding the effects of low and protected doses of ionizing radiation is considerably less than those caused by high intensity, short-term exposure”.

U.S. Congress directs Department of Energy (DOE) to initiate a new program to support the research needed to establish science-based risk assessment standards and guidelines for exposure to low levels of ionizing radiation. DOE creates the Low Dose Radiation Research Program (LDRRP) in 1998. According to the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR), the natural rays from the Sun and the Earth transmit about 2.4 mSv to each individual every year. Human activities exposure us to an additional radiation dose, especially the techniques used in non-invasive medical imaging (radiography, CT scanners) that are becoming increasingly common in the industrialized countries. UNSCEAR estimates that this accounts for an average dose per individual of 1.2 mSv/year. The present impact of Chernobyl, atmospheric nuclear tests and electricity generation by nuclear plants account for very minute proportions, just 0.002, 0.005 and 0.002 mSv respectively (DOE LDRRP, 2007).

Ionizing radiation consists of both particles and electromagnetic radiation. The particles are further classified as electrons, protons, neutrons and alpha particles, depending on their atomic characteristics. The most common electromagnetic radiations with enough energy to produce ions, break chemical bonds and alter biological function are x-rays and gamma rays. Exposure to such radiation can cause cellular and molecular changes such as mutations, chromosome aberrations and cell killing. At high doses, it is well-established that ionizing radiation is capable of increasing the cancer rate in exposed populations at low doses it is not possible to detect changes in cancer frequency. Studies at lower doses are currently being conducted as new tools become available to both deliver the radiation and study the response of cell and molecules. This research resulted in some very interesting results that suggest that low doses of ionizing radiation with matter triggers many biological responses that were not predicted from past experience. These results include bystander effects, change in the spectrum of gene activation, adaptive responses and genomic instability [1, 2].

Investigations of the biological effects of ionizing radiations have revealed that small doses of high energy ions can be hazardous to highly specialized cell system including the brain [3]. On the other hand, opinions differ as to which of the central nervous system structural components, i.e., neurons, glial or endothelial cells, may be primarily damage by the ionizing radiations. Effects of the radiation exposure were studied in relatively simple epithelial systems in animals, e.g., the rabbit’s ear chamber, rat’s subcutaneous air pouch, and rat choroid plexus [4-8].

Choroid plexus of the brain is an ideal model for studying the development of radiation damage due to a close contact between vascular and epithelial cells, which normally have very slow turnover. Choroid plexus plays an integral role in the brain barrier system [9].

With these data in view, we aimed to study the influence of high energy ion exposures on the rat choroid plexus structure. Our investigation describes the ultrastructural and morphometric changes in the rat choroid plexus epithelial and endothelial cells after total-body exposure to low doses of high energy oxygen ions, fast neutrons and gamma rays.

MATERIAL AND METHODS

Three-months aged female Wistar rats were divided into four group: I group – irradiated with single dose of 10⁴ particles/cm² of oxygen ions (n=3), II group – irradiated with fast neutrons (n=3) to 1.5 MeV at the dose of 1.0 Gy, III group – irradiated with gamma rays Co⁶⁰ (n=3) at the dose of 1.0 Gy and IV group – control six months rats (n=4). Three months after irradiation the animals were intracardially perfused. Extracted choroid plexuses were postfixed in 1% OsO₄ in 0.2 M cacodilate buffer, dehydrated through graded ethanol and embedded in Durcupan and examined with JEOL JEM 1200EX transmission electron microscope.

Morphometric analysis

We obtained morphometric data from the light microscope Carl Zeis Jena at 1000x magnification using a square grid system (625 test points) calibrated for linear measurement in µm and stereological measurement in µm².

Statistical analysis

Results are reported as mean values ± SEM and as relative part in percentage, and statistically analyzed by Student’s t-test using statistical package (STATISTICA, ver.6, Stat-Soft Inc., 2001), and differences were regarded as significant at p<0.05.

RESULTS AND DISCUSSION

In contrast to control rat choroid plexus epithelial cells (Fig. 1) on the apical surface of the epithelial cells of the rat choroid plexus 3 months after irradiation with high energy oxygen ions were seen cytoplasmic protrusions, the so-called blebs (Fig. 2). The microvilli were elongated and dilated. On the apical epithelial surface were seen intraventricular macrophages, many vacuoles in the basal epithelial part, and many complex infoldings of the intercellular basal lamina (Fig. 3). The epithelial nuclei were oval with small invaginations (Fig. 4). In the epithelial cytoplasm there were observed well defined Golgi apparatus, dense bodies, vesicles and multivesicular bodies (Fig. 5). Other characteristic ultrastructural change of the choroid plexus epithelial cells was the presence of vacuoles, containing glycogen granules in the apical epithelial cytoplasm (Fig. 6). It is a well known
Fig. 1. Epithelial cell of the rat choroid plexus aged 6 months. X 5 000

Fig. 2. Apical surface of the epithelial cell (EC) of the rat choroid plexus 3 months after irradiation with high energy oxygen ions. The apical end of this choroidal cell shows elongated and dilated microvilli at the apical part (→) and cytoplasmic protrusion (bleb) (*). X 10 000

Fig. 3. Epithelial cell (EC) of the rat choroid plexus 3 months after irradiation with high energy oxygen ions. A/ Intraventricular macrophage (Mac), X 6 000; B/ Basal part – there are many vacuoles (Vac); the intercellular basal lamina with many complex infoldings (→), X 7 500; C/ Fenestrated capillary (*). X 30 000
Fig. 4. Basal part of the epithelial cell (EC) of the rat choroid plexus 3 months after irradiation with high energy oxygen ions. Nucleus (N) exhibiting invagination; basal membrane (bm); connective tissue (*) and capillary lumen (L). X 10 000

Fig. 5. Dark (D) and light (L) epithelial cells of the rat choroid plexus 3 months after irradiation with high energy oxygen ions. In the epithelial cytoplasm there were seen well defined Golgi apparatus (*), dense bodies (→), vesicles (**), multivesicular bodies (⇒), nucleus (N). X 25 000

Fig. 6. Apical part of the epithelial cell (EC) of the rat choroid plexus 3 months after irradiation with high energy oxygen ions. In the epithelium there were seen vacuoles (Vac) containing glycogen granules. X 25 000

Fig. 7. Epithelial cells (EC) of the rat choroid plexus 3 months after irradiation with fast neutrons. In the epithelial cytoplasm were seen well defined Golgi apparatus (⇒), coated vesicles (→), multivesicular bodies (*), pinocytotic vesicles (**), nucleus (N). X 15 000

Fig. 8. Apical part of the epithelial cell (EC) of the rat choroid plexus 3 months after irradiation with gamma rays. There are elongated mitochondria in the cytoplasm (→). X 15 000

Fig. 9. Epithelial cells (EC) of the rat choroid plexus 3 months after irradiation with gamma rays. Lateral intercellular junctions (←), many coated vesicles (cv) and pinocytotic vesicles (*). X 50 000
fact, that glycogen granules in cytoplasm of the choroid plexus epithelial cells are incidental to the fetal and earlier postnatal period of ontogenesis. According to Klatzo et al. [10], the presence of glycogen granules in the epithelial cytoplasm during late ontogenesis can be a sign of a reversible cell alteration or indicate changes in the carbohydrate metabolism.

In the epithelial cytoplasm of the rat choroid plexus 3 months after irradiation with fast neutrons were seen well defined Golgi apparatus, coated vesicles, pinocytotic vesicles and multivesicular bodies (Fig. 7).

Most characteristic ultrastructural changes of the rat choroid plexus 3 months after irradiation with gamma rays were elongated mitochondria, localized at the apical part of the epithelial cells and many pinocytotic vesicles on the basolateral intercellular junctions as well as in the endothelial cells of the capillaries (Fig. 8, 9).

Other ultrastructural changes in the choroid plexus epithelial cells included stimulation of the cell metabolism that could be a compensatory reaction to the changes shortly after the experimental exposures to ionizing irradiation [11]. These ultrastructural changes dominated following the exposure to oxygen ions and gamma rays, when many epithelial cells possessed two nuclei (Fig. 10).

The intracardially perfused marker of cellular permeability, lanthanum nitrate, finds its way into fenestrated capillaries of choroid plexus and interepithelial spaces although it did not overpass the vascular wall of the vessels and the tight junctions (Fig. 11). This observation demonstrates stability of the blood-cerebrospinal fluid barrier of the rat choroid plexus after irradiation with low doses oxygen ions, fast neutrons and gamma rays.

The applied irradiation with oxygen ions, fast neutrons and gamma rays provoked statistically significant changes of the morphometrical parameters of the light and dark epithelial cells in comparison with control ones (Fig. 12). The nuclear area of the light epithelial cells diminished by 4.65% (P<0.01) after irradiation with oxygen ions, while the nuclear area of the dark epithelial cells increased by 6.82% (P<0.01). The cytoplasmic and cell area of both types of epithelial cells increased respectively by 8.86% and 6.62% (P<0.001) of the light cells and by 18.62% and 16.69% (P<0.001) of the dark cells after irradiation with oxygen ions. The results obtained after irradiation with fast neutrons were different. The nuclear, cytoplasmic and cell area of the light and dark epithelial cells diminished respectively by 16.86%, 6.71% and 7.10% (P<0.001) of the light cells and by 10.44%, 12.29% and 12.01% (P<0.001) of the dark cells in comparison with control. The irradiation with gamma rays did not provoke statistically significant differences in the morphometrical data of the light and dark epithelial cells.

The relative number of the light and dark epithelial cells following radiation exposures is shown on the figure 13. Statistically significant differences of the relative part were found after irradiation with oxygen ions (P<0.10) and gamma rays (P<0.02), when the relative part of the dark epithelial cells increased, while the relative part of the light cells decreased after the same irradiations.

Most blood vessels in the plexus choroideus are wide-calibre (10-15 µm) fenestrated capillaries, which include non-fenestrated endothelial segments with some vesicles [12]. Rather extended parts of endothelium almost devoid of vesicles and fenestrae also occur. Arterioles are identified by the close relationship of endothelial cells and smooth muscle cells. The endothelium is higher (about 0.4 µm) than in capillaries (0.3-0.03 µm), and usually contains more vesicles. Venules cannot be identified with certainly, although some exposed membranes without fenestrations are assumed to be venular [13, 14].

Irradiation with therapeutic doses may result in the development of early and late effects in normal tissues.
Extensive experimental studies have shown a clearly defined association between vascular damage and the development of late radiation effects, however, the exact role played by vascular lesions remains uncertain [15, 16].

Changes in the relative number of all blood vessels and vessels divided in four subgroups were determined after irradiation with a single dose of fast neutrons, oxygen ions and gamma rays in comparison to control rats. These findings are shown in Figure 14 and Table 1. The significant changes three months after fast neutrons irradiation were approximately 13% reduction (P<0.001) in the number of vessels of 5-7 µm in diameter and 22% reduction (P<0.001) in vessels of 7-16 µm in diameter. A significant increased of 32% was seen in the number of large vessels of 16-30 µm (P<0.001) and 4% in vessels >30 µm in diameter. Similar changes were
estimated after irradiation with a single dose of high energy oxygen ions in comparison to control rats: approximately 12% reduction (P<0.001) in the number of vessels of 5-7 µm in diameter and 2% reduction in vessels of 7-16 µm in diameter. A significant increase of 7% was found in the number of large vessels of 16-30 µm (P<0.01) and >30 µm (P<0.01) in diameter after irradiation with oxygen ions. Slightly different are the results obtained after exposure to gamma rays, when approximately 10% reduction (P<0.001) in the number of vessels of 5-7 µm and 7-16 µm in diameter was observed.

Significant changes were not seen in luminal diameter and luminal area of all blood vessels after low doses irradiations.

The total-body exposures to low doses fast neutrons and gamma rays provoked similar ultrastructural alterations in the rat choroid plexus. Most of the epithelial cells exhibited ultrastructural signs of increased transcellular transport (coated vesicles, multivesicular bodies, micropinocytotic vesicles) of substances. Similar ultrastructural changes of the rat choroid plexus epithelium were seen after irradiation with high energy carbon ions [17]. Probably, this increased intensity of the cellular transport is related with early post-exposure edema or subserves elimination of toxic substances consequent to radiation exposure. The ultrastructural changes in the choroid plexus epithelial cells after exposure to high energy oxygen ions included increased absorption-secretion activities and stimulation of the cell metabolism that could be a compensatory reaction to the changes shortly after the experimental exposure [11].

The obtained our morphometrical data of rat choroid plexus have shown that the nuclear and cytoplasmic area of both types of epithelial cells is changed statistically significantly after irradiation with oxygen ions and neutrons. Statistically significant differences were found after irradiation with oxygen ions and gamma rays, when the relative part of the dark epithelial cells increased by 8.2% and 12.7% respectively. The obtained morphometrical data have shown that the changes of the choroid plexus epithelial cells are more marked after irradiation with oxygen ions and neutrons in comparison to gamma rays.

In the choroid plexus of rat brain, which has a relatively simple vascular system, significant changes were shown in the morphometrical data of blood vessels after irradiation with small doses of high energy oxygen ions, fast neutrons and gamma rays. It was estimated that the relative part of the capillaries, i.e. vessels <16 µm in diameter were 70.66% in control rats, 34.86% in fast neutrons, 56.34% in oxygen ions and 70.52% in gamma rays irradiated rats. The initial loss of capillaries and the increase in the number of larger vessels in plexus choroideus three months after irradiation were consistent with the effects attributed to regeneration in the choroid plexus. These changes may be indicative of compensatory reactions in the organism following radiation.
exposure. Experimental study has shown a significant reduction in the number of blood vessels >16 µm in diameter and atrophy of the choroid plexus epithelial cells only after 25 Gy of X-rays [16, 18].

**In conclusion**, it can be pointed out that the applied irradiations provoke significant changes in the rat choroid plexus blood vessels. These results clearly demonstrate that the effect on blood vessels after irradiation can be induced in the choroid plexus by single dose of 1.0 Gy fast neutrons, oxygen ions and gamma rays. We suggest a hypothesis that the vascular damage is a predominant factor leading to development of late effects in irradiated normal tissue.

**Table 1. Morphometric data of choroid plexus blood vessels of control rats and neutrons, oxygen ions and gamma rays irradiated rats (luminal diameter in µm, luminal area in µm²)**

<table>
<thead>
<tr>
<th>Blood vessels</th>
<th>Control rats</th>
<th>Irradiated rats</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Luminal Diameter ± SEM</td>
<td>Luminal area ± SEM</td>
</tr>
<tr>
<td>5–7 µm</td>
<td>7.37±0.24</td>
<td>100.92±2.84</td>
</tr>
<tr>
<td>7–16 µm</td>
<td>11.16±0.41</td>
<td>174.13±10.52</td>
</tr>
<tr>
<td>16–30 µm</td>
<td>21.56±1.26</td>
<td>401.28±30.59</td>
</tr>
<tr>
<td>&gt;30 µm</td>
<td>33.87±1.87</td>
<td>731.25±40.49</td>
</tr>
</tbody>
</table>

**Sažetak**

U radu su prikazana ultrastrukturna i morfometrička istraživanja svetlih i tamnih čelija horoi-dnog pleksusa i endotelnih čelija pacova nakon zračenja niskim dozama jonizujućeg zračenja jonima kiseonika visoke energije, neutronima i gama zracima. IZlaganje celog tela niskim dozama brzih neutrona i gama zaracima izaziva slična ultrastrukturna oštećenja horoidnog pleksusa, pacova. Većina epitelnih čelija pokazuje ultrastrukturne znake povećanog trans-čelijskog transporta materija. Ultrastrukturne promene epitelnih čelija horoidnog pleksusa nakon izlaganja jonima kiseonika visoke energije uključuju povećanu apsorpcionu-sekretornu aktivnost i stimulaciju čelijskog metabolizma koja može biti kompenzatorna reakcija na promene kratko posle ekspozicije. Primjenjena iradijacija izaziva statistički značajne promene čelija u horoidnom pleksusu pacova. Dobijeni ultrastrukturni i morfometrički podaci nakon zračenja celog tela pacova sa niskim dozama pokazuju da su promene epitelnih i endotelnih čelija pleksus choroides saže izražene nakon iradijacije sa jonima kiseonika i neutronima u poređenju sa gama-zracima. Ovi rezultati jasno pokazuju da efekat na krvne sudove u horoidnom pleksusu nakon zračenja može biti izazvan sa jednom dozom od 1.0 Gy brzih neutrona, gama-zraka i jona kiseonika. Predlažemo hipotezu da je oštećenje krvnih sudova vodeći faktor u razvoju kasnih efekata kod zračenja tkiva.
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